



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/19	A1	(11) International Publication Number: WO 96/26738 (43) International Publication Date: 6 September 1996 (06.09.96)
(21) International Application Number: PCT/US96/03121		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 1 March 1996 (01.03.96)		
(30) Priority Data: 112834 1 March 1995 (01.03.95) IL		
(71) Applicants (for all designated States except US): YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL). BEN-GURION UNIVERSITY OF THE NEGEV [IL/IL]; P.O. Box 653, 84105 Beer-Sheva (IL).		
(71) Applicant (for MW only): RYCUS, Avigail [US/IL]; 16 Kipnis Street, 76305 Rehovot (IL).		
(72) Inventors; and		
(75) Inventors/Applicants (for US only): WALLACH, David [IL/IL]; 24 Borochov Street, 76406 Rehovot (IL). KOST, Josef [IL/IL]; 54 Hashitah Street, 84965 Omer (IL). ELIAZ, Rom [IL/IL]; 46 Mivtza Horev, 84450 Beer-Sheva (IL).		
(74) Agent: BROWDY, Roger, L.; Browdy and Neimark, Suite 300, 419 Seventh Street N.W., Washington, DC 20004 (US).		

(54) Title: PHARMACEUTICAL COMPOSITIONS FOR CONTROLLED RELEASE OF SOLUBLE RECEPTORS

(57) Abstract

A controlled release pharmaceutical composition includes a biocompatible polymeric material, preferably polyethylene-vinyl acetate or poly(lactic-glucolic acid), having incorporated therein a soluble receptor capable of binding to its ligand and thus affecting the ligand's function. The soluble receptor is preferably the soluble form of TNF α receptor. Such compositions are for use in the treatment of disorders in which neutralization of the deleterious effects of TNF α is required.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

**PHARMACEUTICAL COMPOSITIONS FOR CONTROLLED
RELEASE OF SOLUBLE RECEPTORS**

FIELD OF THE INVENTION

5 The present invention relates to pharmaceutical compositions for controlled delivery of soluble forms of receptors from a polymeric matrix.

BACKGROUND OF THE INVENTION

10 Controlled release systems deliver a drug at a predetermined rate for a definite time period, that may range from days to years. These systems provide advantages over conventional drug therapies. For example, after ingestion or injection of standard dosage forms, the blood level of the drug rises, peaks, and then declines. Since each drug has a therapeutic range above which it is toxic and below which it is ineffective, oscillating drug levels may cause alternating periods of ineffectiveness and toxicity. In contrast, a controlled release preparation maintains the drug in the 15 desired therapeutic range by a single administration. Other potential advantages of controlled release system include: (i) localized delivery of the drug to a particular body compartment, thereby lowering the systemic drug level; (ii) preservation of medications that are rapidly destroyed by the 20 body (this is particularly important for biologically sensitive molecules such as proteins); (iii) reduced need for follow up care; (iv) increased comfort; and (v) improved 25 compliance.

Optimal control of drug release may be achieved by 30 placing the drug in a polymeric material. Polymeric materials generally release drugs by diffusion, chemical reaction, or solvent activation.

The most common release mechanism is diffusion, whereby the drug migrates from its initial position in the 35 polymeric system to the polymer's outer surface and then to the body. Diffusion may occur through a reservoir, in which a drug core is surrounded by a polymer film, or in a matrix, where the drug is uniformly distributed through the polymeric

- 2 -

system. Drugs can also be released by chemical reaction such as degradation of the polymer or cleavage of the drug from a polymer backbone.

Combinations of the above mechanisms are possible.

5 Release rates from polymeric systems can be controlled by the nature of the polymeric material (for example, crystallinity or pore structure for diffusion controlled systems; the hydrolytic lability of the bonds or the hydrophobicity of the monomers for chemically controlled systems) and the design of
10 the system (for example, thickness and shape). The advantage of having systems with different release mechanisms is that each can accomplish different goals.

For many years, controlled release systems were only capable of slowly releasing drugs of low molecular weight (<600). Large molecules, such as proteins, were not considered feasible candidates, because polypeptides were considered too large to slowly diffuse through most polymeric materials, even after swelling of the polymer. The discovery that matrices of solid hydrophobic polymers containing
20 powdered macromolecules enabled molecules of nearly any size to be released for over 100 days permitted controlled delivery of a variety of proteins, polysaccharides, and polynucleotides. See Langer, 1990.

The proteins and polypeptides incorporated up to
25 this date in polymeric materials for controlled release are mainly effector molecules, such as insulin, as opposed compositions for controlled release of molecules that will bind and neutralize effector molecules produced in the human body.

30 Tumor necrosis factor- α (TNF α) is a potent cytokine which elicits a broad spectrum of biological responses. TNF α is cytotoxic to many tumor cells and may be used in the treatment of cancer. TNF α enhances fibroblast growth and acts as a tissue remodeling agent, being thus suitable in wound
35 healing. It further induces hemorrhagic necrosis of transplanted tumors in mice, enhances phagocytosis and cytotoxicity of polymorphonuclear neutrophils, and modulates the expression of many proteins, including lipoprotein lipase,

- 3 -

class I antigens of the major histocompatibility complex, and cytokines such as interleukin-1 and interleukin-6. TNF α has been shown to have an effect against virus, bacteria and multicellular, particularly intracellular, parasites. TNF α appears to be necessary for a normal immune response, but large quantities produce dramatic pathogenic effects. TNF α has been termed "cachectin" since it is the predominant factor responsible for the wasting syndrome (cachexia) associated with neoplastic disease and parasitemia. TNF α is also a major contributor to toxicity in gram-negative sepsis, since antibodies against TNF α can protect infected animals.

TNF α has been shown to be involved in several diseases, examples of which are adult respiratory distress syndrome, pulmonary fibrosis, malaria, infectious hepatitis, tuberculosis, inflammatory bowel disease, septic shock, AIDS, graft-versus host reaction, autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis and juvenile diabetes, and skin delayed type hypersensitivity disorders.

Evidence that some of the effects of TNF α can be detrimental to the host have attracted attention to the mechanisms that regulate TNF α function. The intracellular signals for the response to TNF α are provided by cell surface receptors (herein after TNF-R), of two distinct molecular species, to which TNF α binds at high affinity.

The cell surface TNF-Rs are expressed in almost all cells of the body. The various effects of TNF α , the cytotoxic, growth-promoting and others, are all signalled by the TNF receptors upon the binding of TNF α to them. Two forms of these receptors, which differ in molecular size, 55 and 75 kilodaltons, have been described, and will be called herein p55 and p75 TNF-R, respectively. It should be noted, however, that there exist publications which refer to these receptors also as p60 and p80 TNF-R.

Both receptors for TNF α exist not only in cell-bound, but also in soluble forms, consisting of the cleaved extracellular domains of the intact receptors, derived by proteolytic cleavage from the cell surface forms. These soluble TNF α receptors (sTNF-Rs) can maintain the ability to

- 4 -

bind TNF α and thus compete for TNF α with the cell surface receptors and thus block TNF α activity. The sTNF-Rs thus function as physiological attenuators of the activity of TNF α , safeguarding against its potentially harmful effects.

5 It has, however, also been reported that the sTNF-Rs affect TNF α function also by stabilizing its activity, most likely by preventing dissociation of its bioactive trimeric structure to inactive monomers (Aderka et al., 1992). Thus, the sTNF-Rs may affect TNF α activity in two different ways: either they
10 compete for TNF α with the cell surface receptors and block TNF α deleterious effects, or they act as buffering agents and stabilize TNF α activity.

The two sTNF-Rs, hereinafter p55 sTNF-R and p75 sTNF-R, have been formerly designated TNF Binding Proteins I
15 and II, or TBPI and TBPII, respectively (see, for example, EP 398327, EP 412486, and EP 433900). In the present application we will use both designations: p55 sTNF-R or TBPI and p75 sTNF-R or TBPII; by either designation the proteins are the same.

20 The sTNF-Rs are present constitutively in serum at concentrations that increase significantly in both inflammatory and non-inflammatory disease states. The effect of these proteins may differ, however, depending on their concentrations at the site of TNF α action, the relation of
25 their concentration to the local concentration of TNF α , and the rates at which the sTNF-Rs and TNF α are cleared from the site of TNF α action in relation to the rate of decay of TNF α activity. Dependent on these parameters, the sTNF-Rs may, in different situations, affect the function of TNF α in
30 quite a different manner, either by inhibiting the effects of TNF α , or serving as carriers for TNF α or even augmenting the effects of TNF α by prolonging its function (Aderka et al., 1992).

35 The effectiveness of the sTNF-Rs as anti-TNF α drugs can be affected by a number of different factors: The affinity at which the sTNF-Rs bind TNF α , compared to the affinity of the cell-surface receptors, the accessibility of the soluble receptors to the site of TNF α action and the rate

- 5 -

of the clearance of the soluble receptors and of the complexes which they form with TNF α from the site of TNF α formation. The natural forms of the sTNF-Rs are likely to act in the most physiologically relevant manner. However, a major 5 limitation in their use is their rather rapid clearance from the blood. Several attempts have been made to improve these molecules, examples of which are the so-called chimeric "immunoadhesins", in which the sTNF-Rs are linked to the Fc portion of the immunoglobulin molecule (developed by Hoffman La Roche and Immunex), and "PEGulated" sTNF-Rs, in which the 10 sTNF-Rs are cross-linked through PEG molecules (developed by Synergen). Both approaches result in formation of divalent sTNF-R molecules which have longer clearance time and can bind more effectively to the trivalent TNF α molecule. However, 15 they are quite likely to be more immunogenic than the natural soluble receptors, and the clearance of their complex with TNF α from the circulation may not occur at a sufficient efficiency.

Many harmful effects of TNF α result from chronic 20 formation of this cytokine at certain distinct loci in the body. A major foreseen limitation to the use of soluble forms of TNF receptors for defense against such pathological conditions is the difficulty in maintaining therapeutically effective concentration of the soluble receptors, for 25 prolonged durations, at sites of need.

SUMMARY OF THE INVENTION

It is an object of the present invention to develop novel approaches for therapeutic applications of soluble forms 30 of receptors for affecting the functions of their ligands, e.g., for protection against deleterious effects of their ligands, particularly systems which allow local release of the soluble receptor in the body, at a constant rate and for long duration. These approaches are based on incorporation of the 35 soluble receptor into biocompatible polymeric materials, which are implanted or injected in desired bodily compartments. Matrices of polymers containing the soluble receptor enable

local and controlled release of the soluble receptor, in its natural form.

Any soluble receptor that is capable of binding to and affecting the functions of its ligand, either neutralizing 5 the deleterious effects of its ligand and/or stabilizing or augmenting its activity, is encompassed by the invention. Examples of such soluble receptors are the soluble receptors of hormones and of cytokines, and in a preferred embodiment, the soluble receptor is a soluble TNF α receptor.

10 Thus, another object of the present invention is to provide a novel approach for therapeutic applications of soluble TNF α receptors for affecting TNF α effects, e.g., for protection against TNF effects, involving controlled release systems.

15 The present invention thus provides a pharmaceutical composition for controlled release of a soluble receptor; wherein said soluble receptor is incorporated into a biocompatible polymer matrix.

20 Examples of biocompatible polymeric materials that can be used in the compositions of the invention include, but are not limited to, biocompatible nondegradable polymers selected from the group comprising ethylene-vinyl acetate copolymers (EVAc), silicone rubbers, polysaccharides such as cellulose, polyamides, polyacrylates, polyethylenes, 25 polyurethanes, polyisobutylene, and polyphosphazenes, and biodegradable polymers selected from the group comprising polyesters, polyanhydrides, polyorthoesters, polycaprolactone, pseudopolyaminoacids, polypeptides, gelatin, polylactic acid, polyglycolic acid and poly lactic-glycolic acid (PLGA) 30 copolymers.

In one preferred embodiment, a soluble TNF α receptor (sTNF-R) is incorporated in a suitable polymeric material, such as a polyethylene-vinyl acetate matrix, or into poly(lactic-glycolic acid) microspheres.

35 Pharmaceutical compositions according to the present invention may contain the p55 sTNF-R or p75 sTNF-R. In a preferred embodiment, the composition contains the p55 sTNF-R (TBPI).

- 7 -

In one embodiment, the pharmaceutical compositions of the invention are for use in the treatment of disorders in which protection against the deleterious effects of the ligand, e.g., TNF α , is desired. The polymeric matrices containing the sTNF-Rs are placed in desired bodily compartments, thus allowing maintenance of the sTNF-R in the body constantly and for long duration.

In another embodiment, the pharmaceutical compositions of the invention are for use together with the ligand, e.g., TNF α , in order to stabilize and/or augment TNF α activity, for use in any situation in which a beneficial effect of TNF α is desired. In one aspect of this embodiment, the composition comprises a TNF α /sTNF-R complex, which, by administration at tumor sites, may allow effective local anti-tumor function and little systemic undesired effects of TNF α . In other aspects of this embodiment, the composition comprising the TNF α /sTNF-R complex is useful against viral, bacterial and parasiticidal, particularly intracellular multicellular parasiticidal, infections, and for wound healing.

In another embodiment, the invention comprises treatment of a patient to protect said patient from the deleterious effects of a ligand, e.g., TNF α , which comprises administering to said patient a pharmaceutical composition according to the invention comprising an effective amount of the suitable soluble receptor, e.g., sTNF-R, in controlled release form.

In a further embodiment, the invention relates to treatment of cancer, which comprises administering at the tumor site of a patient in need thereof a pharmaceutical composition of the invention comprising a TNF α /sTNF-R complex in controlled release form.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows particle size effect on cumulative TBPI release. Particles of TBPI + BSA powder at three size ranges were incorporated into ethylene-vinyl acetate copolymer (EVAc) matrices at 30% loading: (i) <75 μ m (closed losanges);

- 8 -

(ii) 75-250 μm (open squares), and (iii) 250-425 μm (closed circles)

Fig. 2 shows loading effect on cumulative TBPI release. EVAc matrices with loadings of TBPI + BSA were made 5 using particle size range of 75-250 μm : (i) 10% loading (closed losanges); (ii) 30% loading (open squares), and (iii) 50% loading (closed circles).

Fig. 3 shows cumulative percent release of soluble TNF α receptor I (TBPI) alone or incorporated together with 10 bovine serum albumin (BSA) from microspheres of: (i) poly lactic-glycolic acid (PLGA) 75:25 (open and closed circles, respectively); (ii) PLGA 50:50 (open and closed squares, respectively; and (iii) poly-L-lactic acid (PLLA) (open and closed triangles, respectively).

Figs. 4a-c show *in vivo* results of Balb nude mice 15 inoculated with TNF-producing Chinese hamster ovary (CHO) cells (CHO/TNF) and treated with TBPI or with mouse anti-TNF monoclonal antibody TNF-1 (Ab), wherein:

Fig. 4a is a graphic representation of average 20 group weight (gr) of Balb nude mice treated with: (i) TBPI in EVAC matrix implanted subcutaneously (closed circles); (ii) TBPI in PLGA 75:25 implant (open circles); (iii) anti-TNF antibody injected once, five days after mice inoculation with CHO/TNF cells (Ab once) (closed squares); (iv) anti TNF 25 antibody injected twice, five days after mice inoculation with CHO/TNF cells and a one week later (Ab twice) (open squares); and (v) control, without any treatment (closed triangles);

Fig. 4b presents the results of an *in vitro* 30 assessment of bioactive TNF α levels in mice sera. The sera was collected from mice bearing TNF α -producing Chinese hamster ovary (CHO) cells, at the indicated time periods after 35 implantation of TBPI in EVAc (closed circles) or of TBPI in PLGA 75:25 (open circles), or injection of anti-TNF α antibody injected once (closed squares) or twice (open squares) as in Fig. 4a, as well as from non treated mice (closed triangles), TNF α bioactivity in the sera was determined by applying them at a dilution of 1:100 to cultured MHA2 cell (a derivative of HeLa cells, 24 hours after seeding them at 3×10^4 cells per

- 9 -

9mM microwell) for 10 hours, in the presence of cycloheximide (25 µg/ml), followed by assessment of cell viability by the neutral red uptake method (Wallach et al., 1984); and

Fig. 4c represents survival curves for mice treated
5 with: (i) TBPI in EVAc implant (open squares); (ii) TBPI in PLGA 75:25 implant (closed circles); (iii) anti-TNF antibody once (open triangles); (iv) anti-TNF antibody twice (closed triangles); and (v) control, without any treatment (open circles).

Fig. 5 shows effect of implantation of EVAc
matrices containing TBPI in different sites of the mouse body
on TBPI concentration release: neck (closed circles), back
(open circles), and stomach (closed squares).

Fig. 6 is a graphic representation of the swelling
15 of the ankle joints (mm) showing progression of arthritis at
different development stages of transgenic Tg 197 arthritic
mice progeny treated with implants of EVAc comprising TBPI
(black columns), EVAc alone (grey columns) or untreated
(control) (white columns).

Fig. 7 is a graphic representation of the swelling
20 of the ankle joints (mm) showing progression of arthritis at
different development stages of transgenic Tg 197 arthritic
mice progeny treated with anti-TNF α antibodies once every week
for the experiment duration (65 days) (black columns) and once
25 a week for two weeks (grey columns) or untreated (control)
(white columns).

Fig. 8 is a graphic representation of the average
group weight (gr) of transgenic Tg 211 mice that express human
TNF α mRNA in T cells, treated with implants of EVAc comprising
30 TBPI (closed circles), EVAc alone (open circles) or untreated
(control) (closed squares).

Fig. 9 is a graphic representation of the average
group weight (gr) of transgenic Tg 211 mice treated with
anti-TNF α antibodies once every week for experiment duration
35 (50 days) (closed triangles), and once a week for 2 weeks
(open triangles) or untreated (control) (closed squares).

Fig. 10 represents survival curves for transgenic Tg
211 mice treated with implants of EVAc comprising TBPI

- 10 -

(closed circles) and EVAc alone (open circles) or untreated (control) (closed squares).

Fig. 11 represents survival curves for transgenic Tg 211 mice treated with anti-TNF α antibodies once every week for 5 65 days (closed circles), and once a week for 2 weeks (open circles) or untreated (control) (closed squares).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides for controlled release of any soluble receptor which can bind to and affect the activity of its ligand, such as neutralizing a ligand that has deleterious effects or stabilizing/augmenting a ligand's activity. Examples of such soluble receptors are the soluble receptors of cytokines, e.g., TNF α , IFN- γ , IL-2, IL-6 and the like.

In a preferred embodiment, the compositions of the invention comprise sTNF-Rs that may be obtained from natural sources, such as human urine (Engelmann et al., 1989; Engelmann et al., 1990; Olson et al., 1989, and Seckinger et al., 1989) or by recombinant techniques (EP 433900; Nophar et al., 1990; Schall et al., 1990, and Loetscher et al., 1990), and then further purified as described, for example, in EP 308378 and EP 398327.

As used herein, the terms "sTNF-Rs", "p55 sTNF-R", "p75 sTNF-R", refer to all sTNFs from natural sources or obtained by recombinant DNA techniques, including but not limited to the TNF Binding Proteins I and II described in EP 308378 and EP 398327.

Polymers that can be used in the present invention include, but are not limited to, biocompatible nondegradable polymers selected from ethylene-vinyl acetate copolymers, silicone rubbers, polysaccharides such as cellulose, polyamides, polyacrylates, polyethylenes, polyisobutylene, polyurethanes and polyphosphazenes, and biodegradable polymers selected from polyesters, polyorthoesters, polycaprolactone, polypeptides, pseudo poly(amino acids), gelatin, polyanhydrides, poly-L-lactic acid, poly-D-lactic acid, poly-D,L-lactic acid, polyglycolic

- 11 -

acid, and copolymers selected from poly-L-lactic acid-glycolic acid, poly-D-lactic acid-glycolic acid, poly-D,L-lactic acid-glycolic acid, poly-L-lactic acid-D-lactic acid, and poly-L-lactic acid-D,L-lactic acid.

5 The polymers preferably used in the present invention are ethylene-vinyl acetate copolymers (EVAc), poly lactic-glycolic acid copolymers (PLGA) and poly-L-lactic acid (PLLA).

A pharmaceutical composition comprising STNF-R 10 incorporated in an EVAc matrix may be prepared from commercially available EVAc, after purification, if necessary, for example, by solvent casting as described by Rhine et al., 1980. These matrices constitute diffusion-controlled systems that allow uniform protein 15 distribution, reproducible kinetics and prolonged release of biologically active STNF-R over a period of weeks or even months. As EVAc is a biocompatible but nondegradable polymer, EVAc matrices are suitable for use in applications in which extended drug delivery is required or desired.

20 As the active principle is released from non-degradable polymeric materials such as EVAc by means of diffusion through channels in the matrix, diffusion is enhanced by higher protein loading. Thus, in a preferred embodiment, the active protein is combined with a neutral 25 protein which does not affect the biological response to the active protein in order to increase the total protein load. Examples of such neutral proteins are bovine serum albumin (althouth this is not preferred for human use), human albumin, myoglobin, hemoglobin, etc.

30 In a pharmaceutical composition according to the invention comprising STNF-R incorporated into a biodegradable polymer selected from the group comprising poly-L-lactic acid, polyglycolic acid, and copolymers thereof, commercially available homopolymers or copolymers of lactic acid and/or 35 glycolic acid are compounded in the form of microspheres, suitable both for application as implants or as injections. The microspheres can be prepared, for example, by the modified solvent evaporation method using a double emulsion as

- 12 -

described by Cohen et al., 1991. As these polymers are biodegradable, the implant can be easily injected, avoiding the use of surgical procedures and the need to remove the device after the drug is depleted. The active agent is
5 released by controllable and predictable release kinetics. The system allows prolonged release of the active agent over a period of weeks or even months.

The pharmaceutical composition of the invention comprising a sTNF-R may be used to neutralize the deleterious effects of TNF α in acute diseases, such as septic shock,
10 graft-versus-host disease (GVHD), malaria, infectious hepatitis, tuberculosis, or in chronic diseases, such as cancer-associated cachexia, chronic GVHD, or in autoimmune diseases, e.g., rheumatoid arthritis, juvenile diabetes,
15 systemic lupus erythematosus and multiple sclerosis, or in skin delayed-type hypersensitivity disorders.

The pharmaceutical composition of the invention may be administered by any mode suitable for controlled delivery of the sTNF-R, either as implants, or as local injections,
20 for example, intra-articular injection given into the synovial fluid of the joint cavities in the treatment of rheumatoid arthritis, or intrathecal injection given into the cerebrospinal fluid in the treatment of multiple sclerosis, or topical formulations, e.g., lotions, for
25 treatment of skin disorders. The compositions for treatment of rheumatoid arthritis may comprise other anti-inflammatory agents, and the compositions for treatment of multiple sclerosis may comprise other agents against multiple sclerosis, e.g., beta-interferon and Copolymer-1 (Cop-1).

30 The composition of the invention comprising sTNF-R will be so designed as to deliver in the body an amount of sTNF-R sufficient for blocking TNF α action. In the case of autoimmune diseases, the composition will be designed such as to deliver an amount of sTNF-R that is sufficient to affect
35 the course and severity of the autoimmune disease and to improve the patient's condition, leading to reduction or remission of the disease. The effective amount will depend on the route of administration, the disease to be treated and the

- 13 -

condition of the patient. Determination of the level of p55 sTNF-R or p75 sTNF-R in the serum or other suitable body fluid of the patient by known methods (for ELISA, see Aderka et al., 1991), may help to establish a suitable dose for said patient, 5 considering that the exogenously administered sTNF-R may complement the endogenously formed sTNF-R in neutralizing the TNF α deleterious activity.

When stabilization of TNF α activity is desired, the composition will comprise a complex of TNF α and sTNF-R, for example, a 1:1 complex, for administration at the tumor site. 10

The invention will now be illustrated in a non-limitative manner by the following examples and the accompanying drawings.

15

EXAMPLES

MATERIALS AND METHODS

Materials. PLGA, 50:50, inherent viscosity (i.v.) of 0.51 dl/g, and 75:25, i.v. of 0.48 dl/g, and PLLA (poly-L-lactic acid), i.v. of 0.64 dl/g (both from PURAC 20 Biochem BV, Holland), were used. Ethylene-vinyl acetate copolymer (EVAc), (40% vinyl acetate) (Dupont), bovine serum albumin (BSA) (Sigma Chemical Co., USA), polyvinylalcohol (PVA) of average molecular weight 77000-79000, 88% hydrolyzed (Aldrich Chemical Co.) and TBPI (p55 sTNF-R) (InterPharm 25 Laboratories Ltd., Israel), were used as received. The anti-TNF α antibody designated TNF-1 is a mouse monoclonal antibody raised against human recombinant TNF α in one of the present inventors' laboratory (D. Wallach).

30

Methods. In vitro release profiles were obtained by placing the drug delivery matrices into phosphate-buffered saline (PBS, pH 7.4) contained in a vial and agitated in a shaker bath. Samples were taken periodically and analyzed for TBPI by Enzyme Linked Immuno Sorbent Assay (ELISA) for TBPI, 35 as described by Aderka et al., 1991.

In vivo efficacy of the polymeric systems was examined on three models: (i) Mice implanted with TNF α -producing tumor cells (CHO/TNF) as described by Oliff et

al., 1987, exhibiting severe cachexia leading to death. (ii) Transgenic Tg197 mice which predictably develop arthritis, as described by Keffer et al., 1991. (iii) Transgenic Tg211 mice that express human TNF α mRNA in T cells and develop marked histologic changes and a lethal wasting syndrome, as described by Probert et al., 1993.

EXAMPLE 1. Controlled release of p55 sTNF-R/TBPI from EVAc matrices

Ethylene-vinyl acetate copolymer (40% vinyl acetate by weight) was dissolved in methylene chloride to give a 10% solution (w/v). TBPI solution was added to bovine serum albumin (BSA) powder dissolved in double distilled water. The mixture was freeze dried (24 hr, at 5 microns Hg and -70°C, Freeze Dryer, Lab Conco) into a powder. TBPI + BSA powder was sieved to give particles of <75, 75-250, or 250-425 μ m. A weighed amount of powder from a single size range was added to 15 ml of the polymer solution in a glass vial, and the mixture was poured quickly into the center of a leveled Teflon mold of disks (1 cm in diameter and 0.5 cm in thickness), which had been cooled previously on dry ice for 5 min. During precooling, the mold was covered with a glass plate to prevent excess frost formation. After the mixture was poured, the mold remained on the dry ice for 10 min, and the mixture froze. (The mold was covered again for the last 7 min of this stage.) The frozen slab was easily pried loose with a cold spatula, transferred onto a wire screen, and kept at -20°C for 2 days. The disk then was dried for 2 more days at room temperature in a desiccator under a mild houseline vacuum (600 mtorr). Drying caused the disks to shrink to ≈0.5 cm in diameter and 0.1-0.2 cm in thickness.

The polymeric delivery systems were immersed in PBS medium. EVAc matrices were prepared as disks of 0.5 cm in diameter and thickness of 1-2 mm containing 22.35 μ g of TBPI in every disk for *in vivo* experiments. For *in vitro* experiments, the disks contained 4.47, 13.41 or 22.35 μ g TBPI (10%, 30% and 50% loading). The release of the TBPI was

detected by ELISA for TBPI as a function of time (Aderka et al., 1991).

Figs. 1 and 2 show the effect of drug particle size and loading on release kinetics from EVAc matrices. Particle size significantly affected the drug release rates. An increase in particle size increased the release rates (Fig. 1). Increase in drug loading uniformly increased drug release rates (Fig. 2). Not only did total drug released increase, but also release rates increased.

Drug particle size and loading markedly affected release kinetics of the macromolecular polymeric delivery systems. Because macromolecules are too large to diffuse through the polymer film, it is possible that sustained release occurs via diffusion through channels in the matrix. The incorporation of the macromolecules during the casting may introduce such channels through which the dissolved drug can diffuse. Release rate increases caused by increases in particle size may result from the formation of larger channels of pores in the polymer matrix. Similarly, increased loadings may provide simpler pathways (lower tortuosity) and greater porosity for diffusion, both of which would facilitate the movement of water or PBS into, and protein out of, the matrix.

EXAMPLE 2. Controlled release of p55 STNF-R (TBPI) from PLGA and PLLA microspheres

Poly (lactic-glycolic acid) (PLGA) or poly-L-lactic acid (PLLA) microspheres, were prepared by a modified solvent evaporation method using a double emulsion as described by Cohen et al., 1991. Briefly, TBPI solution or powder of TBPI and BSA (prepared as described in Example 1 above), which was dissolved in double distilled water, were poured into PLGA or PLLA dissolved in methylene chloride. The mixture was probe sonicated (model VC-250, Sonic & Materials Inc.) for 30 sec to form the first inner emulsion (W₁/O). The emulsion was poured, under vigorous mixing using magnetic bar, into 2 ml aqueous 1% polyvinylalcohol (PVA) saturated with methylene chloride to form the second emulsion ((W₁/O)W₂).

- 16 -

The resulting double emulsion was poured into 200 ml of 0.1% PVA and continuously stirred for 3 hr at room temperature until most of the methylene chloride evaporated, leaving solid microspheres. The microspheres were collected by 5 centrifugation (1000g for 10 min), sized using sieves with apertures of 100 μm and freeze dried (16 hr, Freeze Dryer, Lab Conco) into a powder. Unless specified, studies were done with PLGA with a ratio of 75:25 and 50:50 (L/G) and PLLA.

10 The polymeric delivery systems were immersed in PBS. 0.04 g of PLGA or PLLA microspheres containing 18.2 μg of TBPI were evaluated in each experiment, and the release of TBPI was detected by ELISA as a function of time.

15 Fig. 3 shows the cumulative percent release of TBPI from different PLGA copolymers (50:50 or 75:25) or PLLA microspheres. It can be seen that even after 2200 hours, only 10 percent of the TBPI which was incorporated into the microspheres had been released. It is also shown in this graph that the cumulative percent release of TBPI from polymers where TBPI was added together with BSA (TBP+BSA) 20 (closed circles, squares and triangles) is higher than from samples where TBPI was added without BSA (open circles, squares and triangles).

25 **EXAMPLE 3. TBPI released from polymer matrices retains its biological activity in vivo in mice inoculated with CHO/TNF α cells**

As a way of following chronic deleterious effects of TNF α , a well-known animal model for the cachectic effect of TNF α was used in the experiment, i.e., balb-nude mice implanted with TNF α -producing tumor (CHO/TNF α) cells, as 30 described by Oliff et al., 1987.

Balb-nude mice were inoculated subcutaneously with CHO/TNF α cells (Korn et al., 1988) to achieve cachexia. Inoculations were carried out using freshly trypsinized suspensions of cell lines at a concentration of 1×10^7 35 cells/ml (1 ml/mouse). Injections were performed via 23-gauge needles on plastic 3cc syringes. The cells were injected subcutaneously in the back, neck or abdomen region. Serum

- 17 -

samples were obtained several times every week by tail bleeding. The performance of the polymeric systems, implanted five days after mice inoculation with CHO/TNF α cells, was evaluated following the levels of TBPI and TNF α in blood, 5 weight decrease and mortality.

Polymeric matrices according to Examples 1 and 2 above were implanted or injected in the balb-nude mice five days after mice inoculation subcutaneously with TNF α -producing CHO cells to achieve cachexia and their performance was 10 evaluated following the levels of TBPI and TNF α in blood (ELISA). The parameters examined were: (1) biological activity of TBPI as function of time; (2) comparison of TBPI treatments to treatment with mouse antibodies against human TNF α (raised in the inventors' laboratories and designated 15 TNF-1) injected once (five days after mice inoculation with CHO/TNF α cells, marked as Ab-once) or twice by interval of one week from each other (five days after mice inoculation with CHO/TNF α cells and a one week later, marked as Ab-twice); (3) the progress of cachexia in terms of mice average group weight 20 and mice mortality; and (4) site of implantation (subcutaneous in the neck, back or stomach).

TNF α may cause cachexia, and can induce progressive weight loss in tumor-bearing animals. The *in vivo* efficacy of the matrices was examined in mice implanted with TNF α -producing tumor cells. Mice injected with 1×10^7 CHO/TNF α 25 cells that produce constitutively TNF α at high levels, exhibited severe cachexia and weight loss, leading to death. Mice that, in addition, were implanted with matrices of polymers containing TBPI, increased their body weight without 30 mortality, indicating that the TBPI released from the matrices provided long-lasting protection against TNF α .

To assess the biological activity of TBPI in the tumor-bearing mice, serum samples were collected at various time points and the bioactivity of TNF α in them was estimated 35 by determining their cytotoxicity to MHA2 cells (a derivative of HeLa cells that are sensitive to TNF α). As shown in Fig. 4b, inoculation of the TBPI-containing matrices resulted in prolonged and significant reduction in serum TNF α bioactivity,

indicating that the TBPI released from the matrices inhibits strongly the binding of TNF α to the cell surface TNF α receptors on the MHA2 cells.

For comparison, mice were injected with antibodies to TNF α once (five days after mice inoculation with CHO/TNF α cells, marked as Ab-once) or twice (five days after mice inoculation with CHO/TNF α cells and a one week later, marked as Ab-twice). As shown in Fig. 4a, antibodies affected mice weight only for few days from time of injection (mice maintained their body weight), and then they developed progressive wasting, while TBPI, when incorporated into polymers, was present in the body for long time.

The short duration of the effect of injected anti-TNF α antibodies (TNF-1) is manifested also in the pattern of TNF α bioactivity in the sera of the antibody-injected mice, as shown in Fig. 4b.

In Fig. 4c it can be seen that mice treated with polymeric systems which permit the controlled and localized release of TBPI, survived for more than 50 days, in contrast to mice treated with the TNF-1 antibodies, that did not survive and developed cachexia when the injections of antibodies were stopped.

These results indicate that treatment based on polymeric controlled delivery of TBPI prevents development of the wasting syndrome, and might be preferred to injection of antibodies to TNF α , since it eliminates the need to inject every week and provides longer-lasting protection against TNF α . Moreover, being natural molecules, the soluble TNF α receptors are more suitable for prolonged use. Anti-TNF α antibodies will sooner or later raise antibodies against them, which will prevent their action, and are therefore not suitable for prolonged use. This is true also for human or humanized antibodies, that will raise anti-idiotypic antibodies that will block their function.

There is no significant difference in TBPI release kinetics from matrices of EVAc implanted in the mice neck, back or stomach, as function of implantation site of the

matrices (Fig. 5). The TBPI release kinetics is constant along all the release duration.

5 **EXAMPLE 4. TBPI release from polymer matrices retains its biological activity in vivo in transgenic arthritic Tg197 mice**

The performance of EVAc matrices made in accordance with Example 1 (implanted subcutaneously by minor surgery 14 days from birth) in transgenic Tg197 mice was evaluated following the levels of swelling of the ankle joints. The disease was evident at around 4-6 weeks of age with swelling of the ankle joints. Additional swelling of the ankle joints and impairment in leg movement progressed to complete loss of movement of the hind legs at around 9-10 weeks of age. Moreover, progressive weight loss was a common feature in these mice. Treatment of these arthritic mice with EVAc matrices containing TBPI, however, completely prevented swelling of the ankle joints (Fig. 6). Moreover, treated animals developed normally and showed no signs of arthritis or weight loss even past 10 weeks of age.

20 For comparison, mice were injected 14 days from birth with TNF-1 antibodies once every week for all treatment duration (65 days), or once a week for 2 weeks. As shown in Fig. 7, the antibodies affected swelling only if given once every week for all the experiment duration (black columns).

25 Mice that were injected every week developed normally and showed no signs of arthritis or weight loss even past 10 weeks of age, when impairment of movement is a common macroscopial feature in these mice. However, mice that were injected once a week for 2 weeks developed swelling of the ankle joints and impairment in leg movement progressed to complete loss of movement of the hind legs at around 9-10 weeks of age with progressive weight loss.

30 These results indicate that treatment based on polymeric controlled delivery of TBPI completely prevents development of arthritis in mice, and might be preferred to injection of antibodies to hTNF α , since it provides

longer-lasting protection against TNF α and eliminates the need of injection every week.

EXAMPLE 5. TBPI released from polymeric matrices retains its biological activity in vivo in transgenic Tg211 mice

Transgenic Tg211 mice that express human TNF mRNA in T cells developed marked histologic changes and a lethal wasting syndrome. The performance of the polymeric systems containing TBPI, implanted 14 days from birth, was evaluated following the weight decrease and mortality. The individual animals were weighed several times every week. EVAc matrices were implanted subcutaneously by minor surgery.

As shown in Figs. 8 and 10, untreated mice (control group: closed squares) developed severe progressive weight loss, leading to death, while mice that were implanted with EVAc matrices containing TBPI (closed circles), increased their body weight and survived for all experiment duration.

For comparison, mice were injected 14 days from birth with TNF-1 antibodies once every week for all experiment duration, or once a week for 2 weeks. As shown in Fig. 9, administration of anti-TNF α antibodies completely prevented development of the wasting syndrome if given from birth every week. However, if given only once a week for 2 weeks, they were not effective, and mice developed the wasting syndrome which lead to death, as shown in Fig. 11.

These results indicate that treatment based on polymeric controlled delivery of TBPI completely prevents development of the wasting syndrome in mice, and might be preferred to injection of antibodies to hTNF α .

The *in vivo* results indicate that TBPI released retains its biological activity, as shown by the average group weight change (Fig. 4a) and the high percent of live MHA2 cells that are sensitive to TNF α (Fig. 4b).

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by

- 21 -

reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

5 Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

10 The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various 15 applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the 20 teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the 25 teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

30

35

References:

1. Aderka D. et al., Cancer Res., 51:5602-5607 (1991).
2. Aderka D. et al., J. Exp. Med., 175:323-329 (1992).
3. Cohen S. et al., Pharm. Res., 8:713-720 (1991).
4. Engelmann H. et al., J. Biol. Chem., 264:11974-80 (1989).
5. Engelmann H. et al., J. Biol. Chem., 265:1531-36 (1990).
6. Korn J.H. et al., Lymphokine Res., 7:349-358 (1988).
7. Langer R., Science, 249:1527-1533 (1990).
8. Loetscher H. et al., Cell, 61:351-359 (1990).
9. Nophar Y. et al., EMBO J., 9:3269-78 (1990).
10. Oliff A. et al., Cell, 50:555-563 (1987).
11. Olson I. et al., Eur. J. Haematol., 42:270-75 (1989).
12. Probert L. et al., J. Immunol., 151:1894-1906 (1993).
13. Rhine W.D. et al., J. Pharm. Sci., 69:265-270 (1980).
14. Schall T.J. et al., Cell, 61:361-370 (1990).
15. Seckinger P. et al., J. Biol. Chem., 264:11966-973 (1989).
16. Wallach D. et al., J. Immunol., 132:2464-69 (1984).

CLAIMS:

1. A pharmaceutical composition for controlled release of a soluble receptor capable of binding to its ligand and thus affecting the ligand's function, comprising a biocompatible polymeric material having said soluble receptor incorporated therein.

2. A pharmaceutical composition according to claim 1, wherein said polymeric material is a biocompatible nondegradable polymer selected from the group consisting of ethylene-vinyl acetate copolymers (EVAc), silicone rubbers, polysaccharides, polyamides, polyacrylates, polyethylenes, polyurethanes, polyisobutylene, and polyphosphazenes.

3. A pharmaceutical composition according to claim 1, wherein said polymeric material is a biodegradable polymer selected from the group consisting of polyesters, polyanhydrides, polyorthoesters, polycaprolactone, pseudopolyaminoacids, polypeptides, gelatin, polylactic acid, polyglycolic acid and poly(lactic-glycolic acid) copolymers (PLGA).

4. A pharmaceutical composition according to any one of claims 1 to 3, wherein said soluble receptor is a soluble receptor of a hormone or a cytokine.

5. A pharmaceutical composition according to claim 4, wherein said soluble receptor is the soluble form of a TNF α receptor.

6. A pharmaceutical composition according to claim 2, wherein said soluble receptor is the soluble form of a TNF α receptor.

7. A pharmaceutical composition according to claim 6, wherein the polymer is polyethylene-vinyl acetate.

8. A pharmaceutical composition according to claim 3, wherein said soluble receptor is the soluble form of a TNF α receptor.

9. A pharmaceutical composition according to claim 8, wherein the polymer is poly(lactic-glycolic acid).

10. A pharmaceutical composition according to claim 5, wherein the soluble TNF α receptor is the soluble p55 TNF α receptor (p55 STNF-R).

11. A pharmaceutical composition according to claim 5, wherein the soluble TNF α receptor is the soluble p75 TNF α receptor (p75 STNF-R).

12. A pharmaceutical composition according to claim 5, for the treatment of disorders in which neutralization of the deleterious effects of TNF α is required.

13. A pharmaceutical composition according to claim 12, for the treatment of septic shock.

14. A pharmaceutical composition according to claim 12, for the treatment of cancer-associated cachexia.

15. A pharmaceutical composition according to claim 12, for the treatment of autoimmune diseases.

16. A pharmaceutical composition according to claim 15, for the treatment of rheumatoid arthritis.

17. A pharmaceutical composition according to claim 16, further comprising an anti-inflammatory agent.

18. A pharmaceutical composition according to claim 15, for the treatment of multiple sclerosis.

- 25 -

19. A pharmaceutical composition according to claim 18, comprising a further agent for the treatment of multiple sclerosis, such as beta-interferon or Copolymer-1.

20. A pharmaceutical composition according to claim 15, for the treatment of systemic lupus erythematosus.

21. A pharmaceutical composition according to claim 12, for the treatment of graft-versus-host reaction.

22. A pharmaceutical composition according to claim 12, for the treatment of skin delayed-type hypersensitivity disorders.

23. A pharmaceutical composition according to claim 7, in the form of implant.

24. A pharmaceutical composition according to claim 9, in the form of implant.

25. A pharmaceutical composition according to claim 9, in the form of injection.

26. A pharmaceutical composition according to any one of claims 23 to 25, for local injection into the synovial fluid for the treatment of joint inflammation in rheumatoid arthritis.

27. A pharmaceutical composition according to any one of claims 23 to 25, for intrathecal injection into the cerebrospinal fluid for treatment of multiple sclerosis.

28. A pharmaceutical composition according to claim 22, for topical application.

29. A pharmaceutical composition according to claim 5, further comprising TNF α .

- 26 -

30. A pharmaceutical composition according to claim 29, for enhancing the beneficial effects of TNF α .

31. A pharmaceutical composition according to claim 30, for the treatment of tumors.

32. A pharmaceutical composition according to claim 30, for the treatment of viral, bacterial and multicellular parasiticidal infections.

33. A pharmaceutical composition according to claim 30, for use in wound healing.

34. A method for the treatment of disorders in which neutralization of the deleterious effects of TNF α is required, comprising administering a controlled release pharmaceutical composition in accordance with claim 5 capable of releasing an effective amount of said soluble form of TNF α receptor.

35. A method in accordance with claim 34, wherein said disorder is selected from the group consisting of septic shock, cancer-associated cachexia, an autoimmune disease, graft-versus-host reaction and skin delayed-type hypersensitivity disorders.

36. A method in accordance with claim 34, wherein said administering step comprises implanting said controlled release pharmaceutical composition.

37. A method for the treatment of a disorder requiring the enhancement of the beneficial effects of TNF α , comprising administering a controlled release pharmaceutical composition in accordance with claim 5 capable of releasing an effective amount of the soluble form of TNF α receptor and TNF α .

Fig. 1

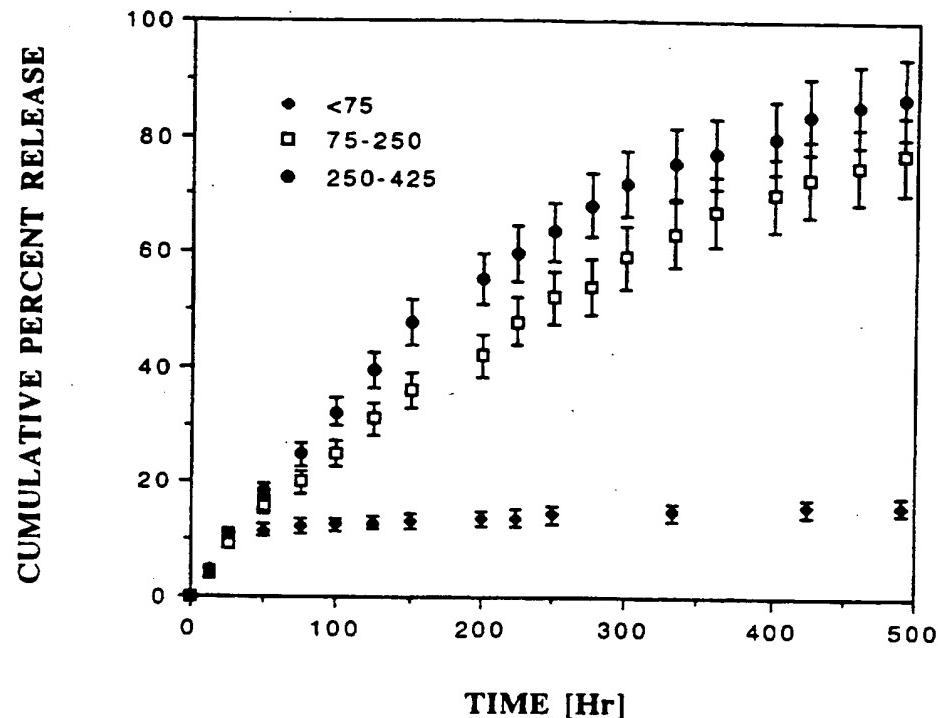
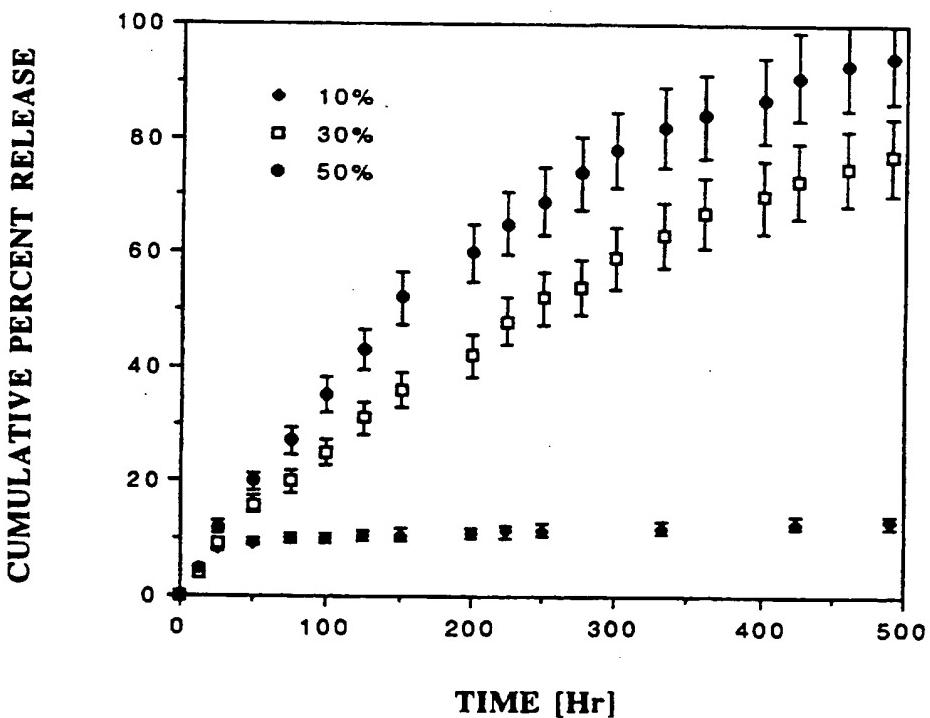


Fig. 2



2 / 7

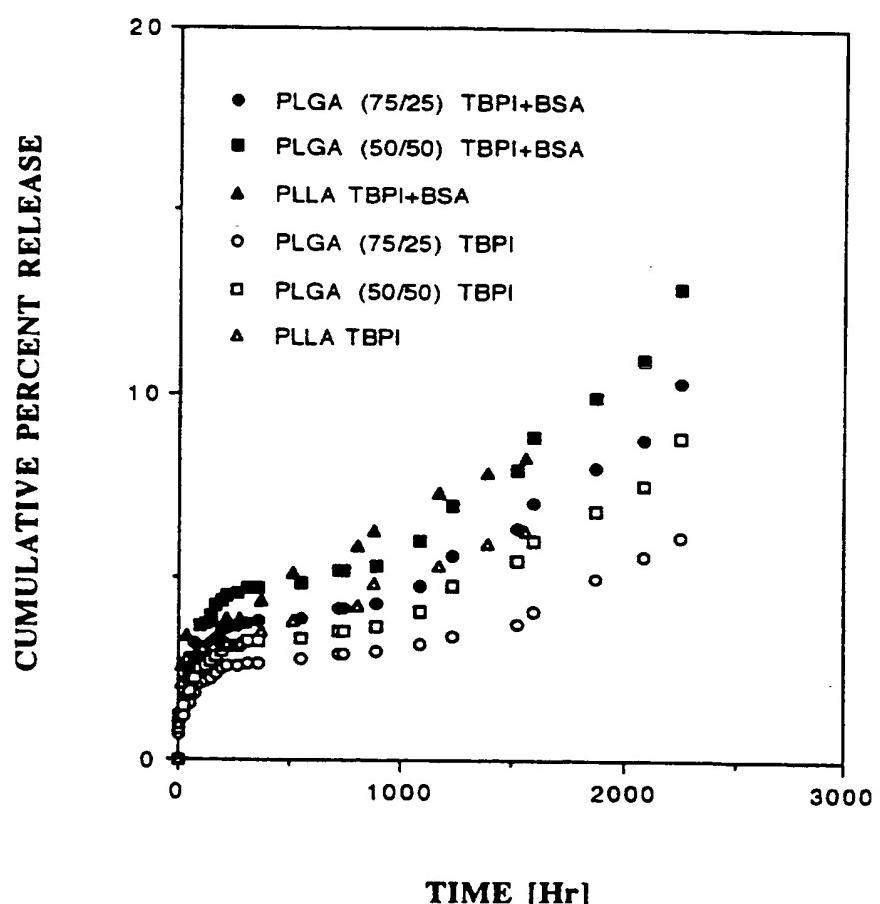
Fig. 3

Fig. 4a

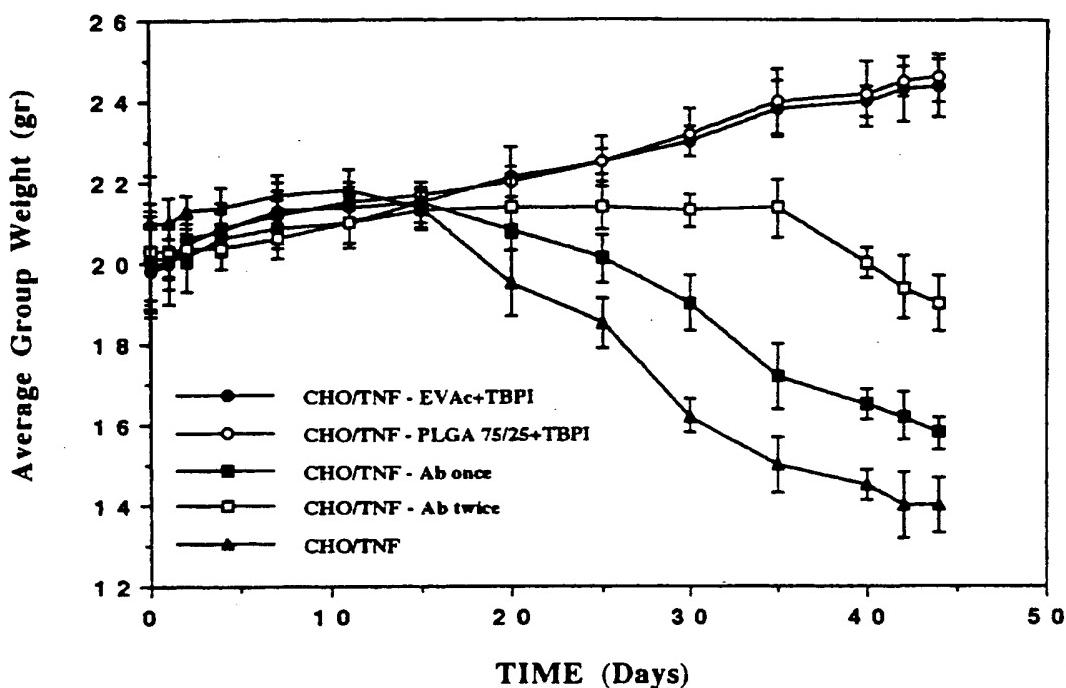
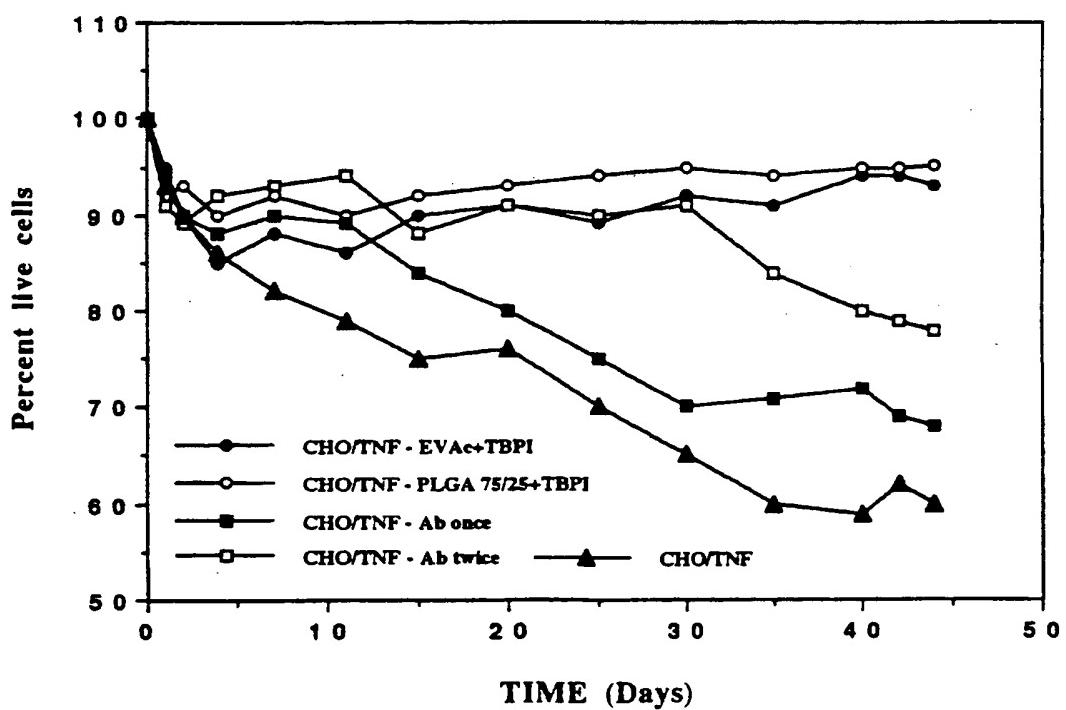


Fig. 4b



4 / 7

Fig. 4c

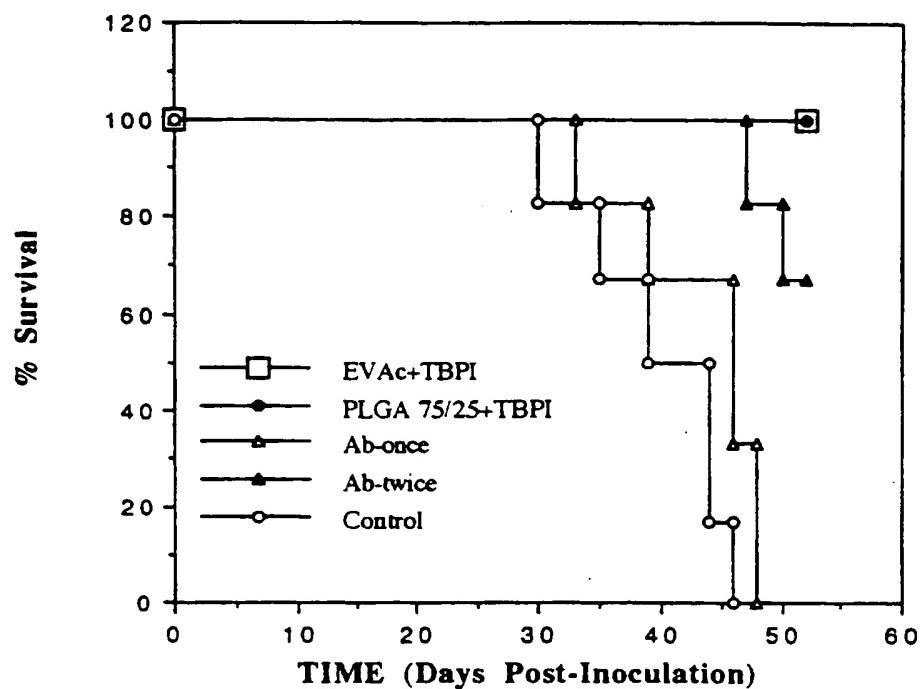
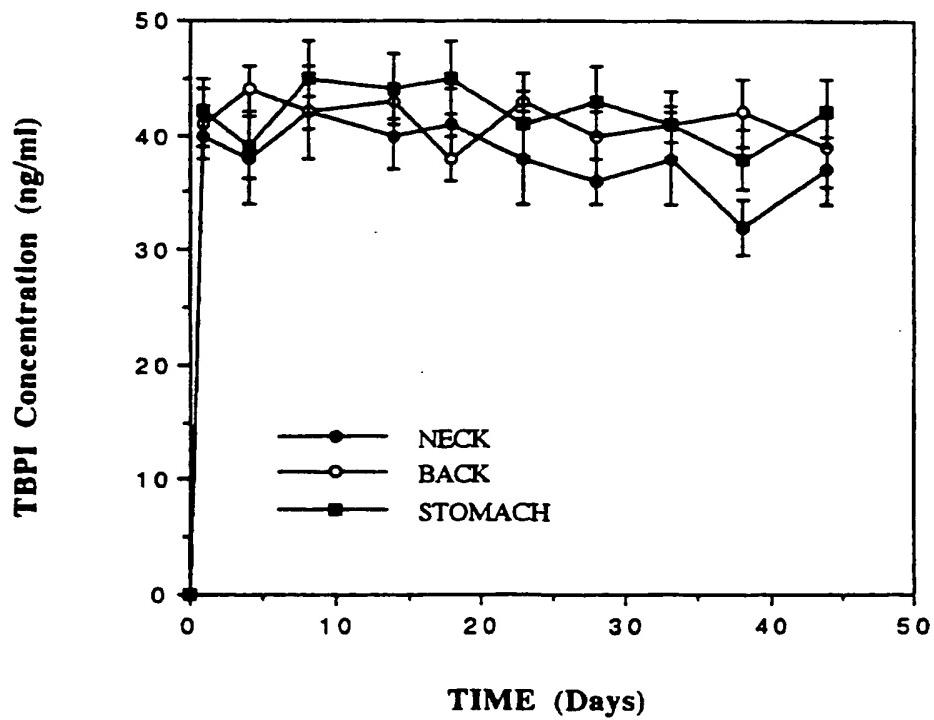


Fig. 5



5 / 7

Fig. 6

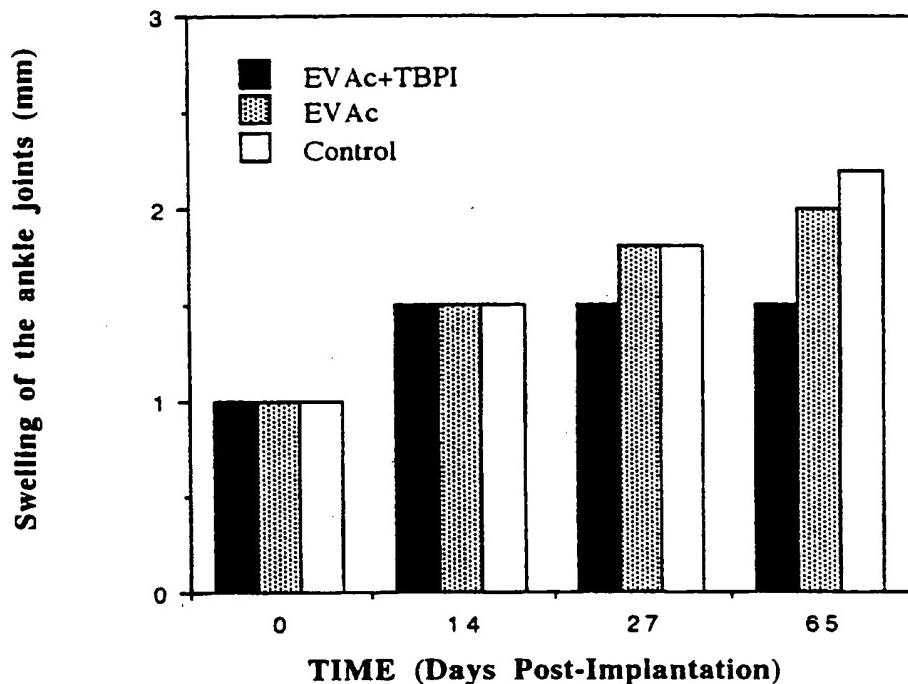
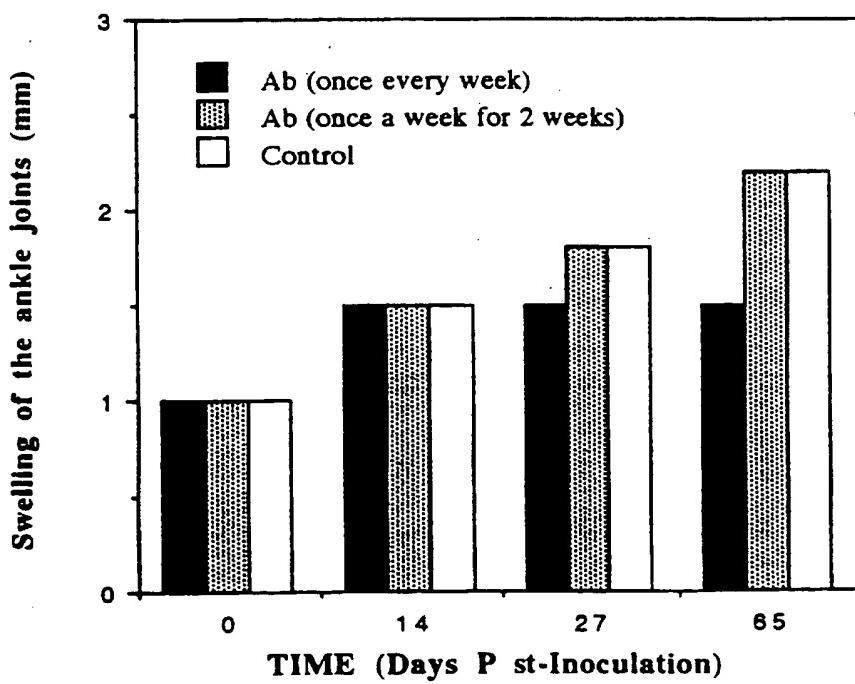


Fig. 7



6 / 7

Fig. 8

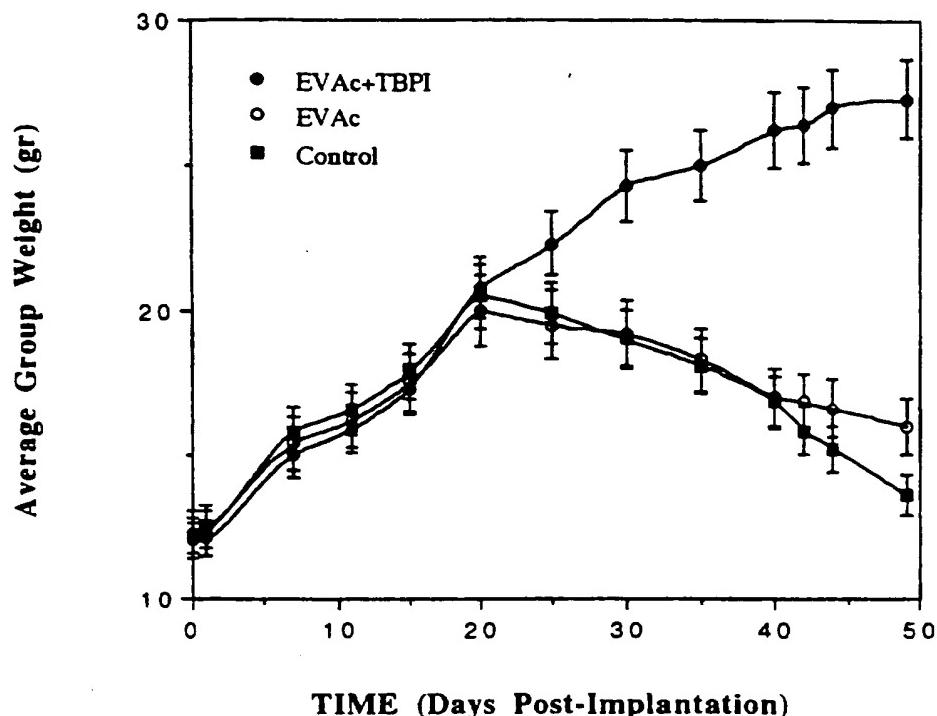
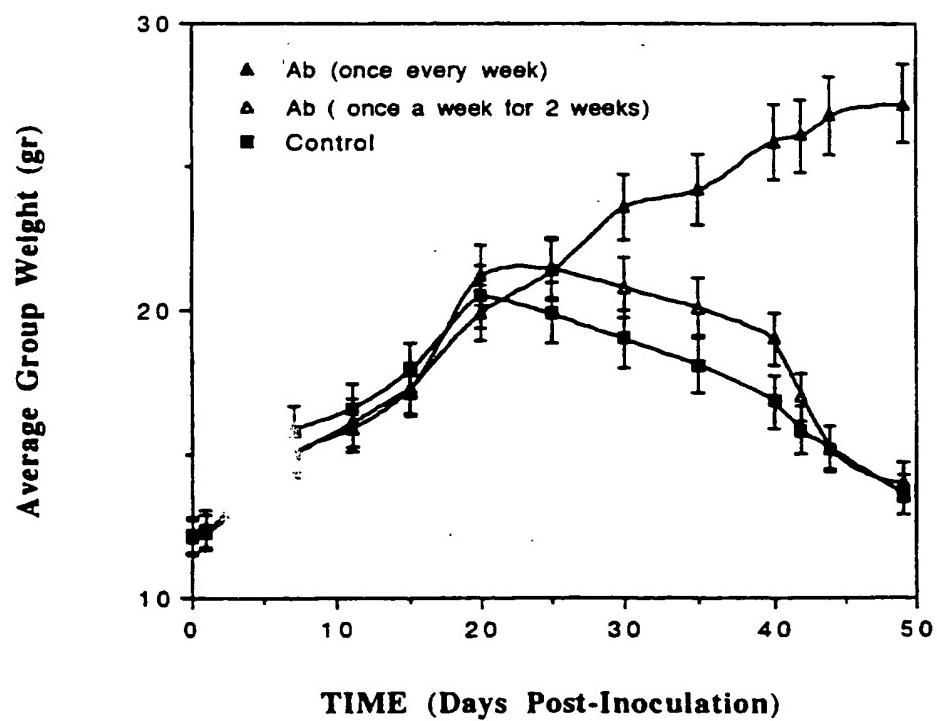


Fig. 9



7 / 7

Fig. 10

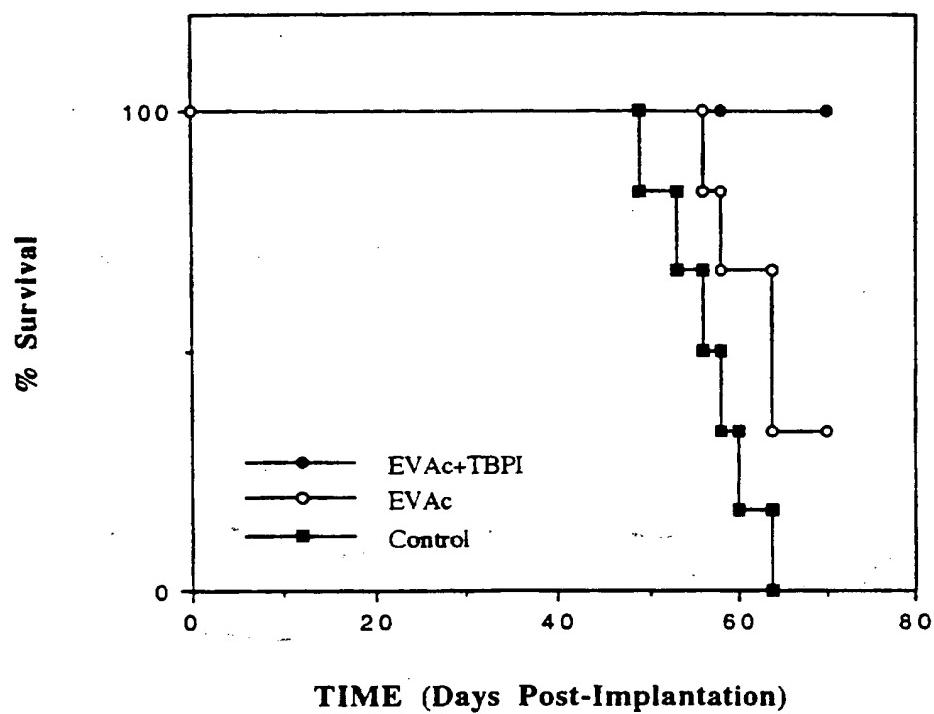
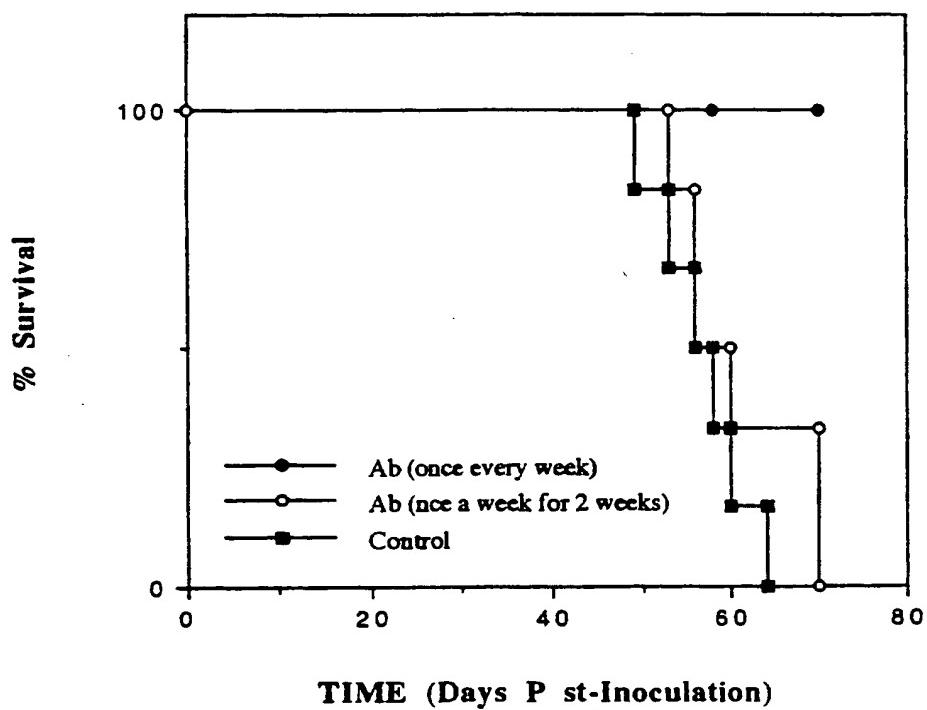


Fig. 11



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/03121

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/19

US CL : 424/85.2; 514/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.2; 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN

search terms: TNF, Tumor Necrosis Factor, control release, EVA, ethylene vinyl acetate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,929,442 A (POWELL) 29 May 1990 (29.05.90), column 5, lines 10-24; column 8, lines 20-24.	1-37
Y	US 4,883,666 A (SABLE ET AL.) 28 November 1989 (28.11.89), whole document	1-37
X, P	US 5,470,582 A (SUPERSAXO ET AL.) 28 November 1995 (28.11.95), whole document	1-37

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date other than the priority date claimed		

Date of the actual completion of the international search
08 JULY 1996Date of mailing of the international search report
15 JUL 1996Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

PATRICK DELANEY

Facsimile No. (703) 305-3230

Telephone No. (703) 308-0196